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     (FILE 'HOME' ENTERED AT 11:44:07 ON 05 NOV 2001)

     FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT
     11:45:08 ON 05 NOV 2001
L1        10969 S AUTOCATALYTIC OR AUTOCATALYTICALLY
L2         1181 S L1 AND (PROTEASE OR PROTEINASE)
L3          142 S L2 AND FUSION
L4            2 S L3 AND CHYMOSIN
L5           61 DUP REM L3 (81 DUPLICATES REMOVED)
L6           39 S L5 NOT PY>1997

     FILE 'CAPLUS' ENTERED AT 11:53:51 ON 05 NOV 2001
                E MOLONEY M/AU 25
L7            1 S (E3 OR E16 OR E17 OR E18) AND (AUTOCATALYTIC OR AUTOCATALYTIC
                E ALCANTARA J/AU 25
L8            1 S (E3 OR E10) AND (AUTOCATALYTIC OR AUTOCATALYTIC?)
                E ROOIJEN G V/AU 25
L9            0 S (E17) AND (AUTOCATALYTIC OR AUTOCATALYTIC?)

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Updated Search

L7 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:728582 CAPLUS
DOCUMENT NUMBER: 130:11268
TITLE: Manufacture of proteins as fusion products with zymogen propeptides for processing of fusion products
INVENTOR(S): **Moloney, Maurice**; Alcantara, Joenel; Van Rooijen, Gijs
PATENT ASSIGNEE(S): Sembiosys Genetics Inc., Can.
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9849326	A1	19981105	WO 1998-CA398	19980423
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9870240	A1	19981124	AU 1998-70240	19980423
EP 977873	A1	20000209	EP 1998-916746	19980423
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
BR 9809416	A	20000613	BR 1998-9416	19980423
ZA 9803471	A	19981027	ZA 1998-3471	19980424

PRIORITY APPLN. INFO.: US 1997-44254 P 19970425
WO 1998-CA398 W 19980423

AB An improved method for manuf. of proteins in a foreign host as a fusion product is described. The method involves synthesis of the protein as a fusion product with a pro-peptide of an **autocatalytically** maturing zymogen that does not process itself in the expression host but that will process in the target organism. The fusion protein can then be administered to the host where it will be processed to release the protein of interest. This avoids the need to purify the fusion protein, cleave it and sep. the cleavage products. Alternatively, an expression construct for the fusion protein can be introduced directly into the target organism. The pro-peptide-polypeptide fusion protein can be cleaved and the recombinant polypeptide released under the appropriate conditions, e.g as a feed additive that is activated in the stomach. A chimeric gene for a fusion protein of hirudin, glutathione-S-transferase and the pro-peptide of chymosin B was constructed using the pGEX expression system and the protein manufd. in Escherichia coli. A significant fraction (5-10%) of the protein accumulated in the cytoplasm with the remainder in inclusion bodies. Incubation of the sol. fraction with chymosin at pH 4.5 resulted in the appearance of an anti-thrombin activity. Incubation at pH 2.0 did not lead to processing of the fusion protein. A fusion protein of prochymosin and carp growth hormone was accurately processed by exts. of the gut of the red turnip beetle Entomoscelis americana.

REFERENCE COUNT: 2
REFERENCE(S): (1) Genex Corp; EP 0134662 A 1985 CAPLUS
(2) Univ Technologies Int; WO 9621029 A 1996 CAPLUS

NUE? Y/(N):y

L6 ANSWER 1 OF 39 MEDLINE
ACCESSION NUMBER: 1998299904 MEDLINE
DOCUMENT NUMBER: 98299904 PubMed ID: 9636275
TITLE: Production of recombinant bovine enterokinase catalytic subunit in Escherichia coli using the novel secretory **fusion** partner DsbA.
AUTHOR: Collins-Racie L A; McColgan J M; Grant K L; DiBlasio-Smith E A; McCoy J M; LaVallie E R
CORPORATE SOURCE: Genetics Institute, Inc, Cambridge, MA 02140, USA.
SOURCE: BIO/TECHNOLOGY, (1995 Sep) 13 (9) 982-7.
Journal code: AL1; 8309273. ISSN: 0733-222X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: B
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980731
Last Updated on STN: 19980731
Entered Medline: 19980721
AB Enterokinase (EK) is a heterodimeric serine **protease** which plays a key role in initiating the proteolytic digestion cascade in the mammalian duodenum. The enzyme acts by converting trypsinogen to trypsin via a highly specific cleavage following the pentapeptide recognition sequence (Asp)4-Lys. This stringent site specificity gives EK great potential as a **fusion** protein cleavage reagent. Recently, a cDNA encoding the catalytic (light) chain of bovine enterokinase (EKL) was identified, characterized, and transiently expressed in mammalian COS cells. We report here the production of EKL in Escherichia coli by a novel secretory expression system that utilizes E. coli DsbA protein as an N-terminal **fusion** partner. The EKL cDNA was fused in-frame to the 3'-end of the coding sequence for DsbA, with the two domains of the **fusion** protein separated by a linker sequence encoding an enterokinase recognition site. Active, processed recombinant EKL (rEKL) was generated from this **fusion** protein via an **autocatalytic** cleavage reaction. The enzymatic properties of the bacterially produced rEKL were indistinguishable from the previously described COS-derived enzyme. Both forms of rEKL were capable of cleaving peptides, polypeptides and trypsinogen with the same specificity exhibited by the native heterodimeric enzyme purified from bovine duodena. Interestingly, rEKL activated trypsinogen poorly relative to the native heterodimeric enzyme, but was superior in its ability to cleave artificial **fusion** proteins containing the (Asp)4-Lys recognition sequence.

L6 ANSWER 2 OF 39 MEDLINE
ACCESSION NUMBER: 97263794 MEDLINE
DOCUMENT NUMBER: 97263794 PubMed ID: 9108151
TITLE: HIV-1 **protease** inhibits its homologous reverse transcriptase by protein-protein interaction.
AUTHOR: Bottcher M; Grosse F
CORPORATE SOURCE: Institut fur Molekulare Biotechnologie, Abteilung Biochemie, Postfach 100 813, D-07708 Jena, Germany.
SOURCE: NUCLEIC ACIDS RESEARCH, (1997 May 1) 25 (9) 1709-14.
Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970609
Last Updated on STN: 19970609
Entered Medline: 19970529
AB The reading frame of the HIV-1 pol gene, encoding **protease** (PR) and reverse transcriptase (RT), including RNase H as well as integrase, was fused to the bacterialbeta-galactosidase gene and overexpressed in Escherichia coli cells. The resulting **fusion** protein was cleaved **autocatalytically** leading to PR, RT and integrase. Immunoprecipitations of bacterial crude extracts with anti-RT antibodies precipitated both RT and PR. Co-precipitation of PR and RT was also observed with anti-PR antibodies, strongly suggesting a physical interaction between fully processed RT and PR within the bacterial cell. Physical interactions were confirmed with purified components by means of an ELISA assay. Furthermore, purified PR inhibited the DNA synthesis activity of purified RT, while its RNase H activity remained unaffected. The type of inhibition was uncompetitive with respect to poly(rA).oligo(dT); the inhibition constant was 50-100 nM. A possible physiological significance of this type of interaction is discussed.

L6 ANSWER 3 OF 39 MEDLINE
ACCESSION NUMBER: 97213978 MEDLINE
DOCUMENT NUMBER: 97213978 PubMed ID: 9060667

TITLE: Identification of active-site residues in **protease** 3C of hepatitis A virus by site-directed mutagenesis.
AUTHOR: Gosert R; Dollenmaier G; Weitz M
CORPORATE SOURCE: Institute for Clinical Microbiology and Immunology, St. Gallen, Switzerland.
SOURCE: JOURNAL OF VIROLOGY, (1997 Apr) 71 (4) 3062-8.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970424
Last Updated on STN: 20000303
Entered Medline: 19970411

AB Picornavirus 3C proteases (3Cpro) are cysteine proteases related by amino acid sequence to trypsin-like serine proteases. Comparisons of 3Cpro of hepatitis A virus (HAV) to those of other picornaviruses have resulted in prediction of active-site residues: histidine at position 44 (H44), aspartic acid (D98), and cysteine (C172). To test whether these residues are key members of a putative catalytic triad, oligonucleotide-directed mutagenesis was targeted to 3Cpro in the context of natural polypeptide precursor P3. **Autocatalytic** processing of the polyprotein containing wild-type or variant 3Cpro was tested by in vivo expression of vaccinia virus-HAV chimeras in an animal cell-T7 hybrid system and by in vitro translation of corresponding RNAs. Comparison with proteins present in HAV-infected cells showed that both expression systems mimicked authentic polyprotein processing. Individual substitutions of H44 by tyrosine and of C172 by glycine or serine resulted in complete loss of the virus-specific proteolytic cascade. In contrast, a P3 polyprotein in which D98 was substituted by asparagine underwent only slightly delayed processing, while an additional substitution of valine (V47) by glycine within putative protein 3A caused a more pronounced loss of processing. Therefore, apparently H44 and C172 are active-site constituents whereas D98 is not. The results, furthermore, suggest that substitution of amino acid residues distant from polyprotein cleavage sites may reduce proteolytic activity, presumably by altering substrate conformation.

L6 ANSWER 4 OF 39 MEDLINE
ACCESSION NUMBER: 97066895 MEDLINE
DOCUMENT NUMBER: 97066895 PubMed ID: 8910297
TITLE: Functional characterization of the prodomain of interleukin-1beta-converting enzyme.
AUTHOR: Van Criekinge W; Beyaert R; Van de Craen M; Vandenabeele P; Schotte P; De Valck D; Fiers W
CORPORATE SOURCE: Laboratory of Molecular Biology, Flanders Interuniversity Institute for Biotechnology and University of Ghent, B-9000 Ghent, Belgium.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Nov 1) 271 (44) 27245-8.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 20000303
Entered Medline: 19961226

AB Interleukin-1beta-converting enzyme (ICE) has been identified as the main **protease** responsible for maturation of the prodomain of interleukin-1beta. Recently, it was shown to belong to a larger gene family, members of which play an important role in programmed cell death. A common feature of the ICE family proteases is the presence of a prodomain that has been hypothesized to keep the enzyme in an inactive form. Expression analysis in yeast revealed **autocatalytic** degradation of p45ICE, but not of p30ICE lacking a prodomain. We further demonstrate that p45ICE, in which the critical cysteine has been mutated, is still able to dimerize in vivo. Dimerization requires the prodomain and occurs prior to autoprocessing. These results provide evidence for a regulatory role of the prodomain of ICE.

L6 ANSWER 5 OF 39 MEDLINE
ACCESSION NUMBER: 97055419 MEDLINE
DOCUMENT NUMBER: 97055419 PubMed ID: 8899706
TITLE: Processing of the AIDA-I precursor: removal of AIDAc and evidence for the outer membrane anchoring as a beta-barrel structure.
AUTHOR: Suhr M; Benz I; Schmidt M A
CORPORATE SOURCE: Institut fur Infektiologie, Zentrum fur Molekularbiologie, Entzundung (ZMBE), Munster, Germany.
SOURCE: MOLECULAR MICROBIOLOGY, (1996 Oct) 22 (1) 31-42.

Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X65022
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970305
Last Updated on STN: 20000303
Entered Medline: 19970219

AB The AIDA-I adhesin known to be responsible for the diffuse adherence (DA) phenotype of the diarrhoeagenic Escherichia coli (DAEC) strain 2787 has been shown previously to be synthesized as a precursor protein and to undergo additional C-terminal processing. Here, the C-terminal processing of the AIDA-I precursor and the outer membrane topology of the cleaved C-terminal fragment, AIDAc, were investigated. By isolation of the cleaved AIDAc fragment and N-terminal sequencing, the C-terminal cleavage site was identified between Ser-846 and Ala-847 thereby indicating a molecular mass of 47.5 kDa for AIDAc. The correct processing to AIDA-I and AIDAc in OmpT, OmpP and DegP **protease**-deficient E. coli strains as well as in avirulent salmonellae and shigellae points to an **autocatalytic** cleavage mechanism. The cleaved AIDAc was localized in the outer membrane. A leader sequence-AIDAc **fusion** was efficiently routed to the outer membrane. Analysis by **protease** digestion, secondary-structure prediction and modelling, by comparison with structurally related bacterial proteins like the IgA1 **protease** from neisseria, the vacuolating toxin from Helicobacter pylori, and the VirG protein of Shigella flexneri, strongly indicates that AIDAc is present in the outer membrane as a beta-barrel structure.

L6 ANSWER 6 OF 39 MEDLINE
ACCESSION NUMBER: 97015916 MEDLINE
DOCUMENT NUMBER: 97015916 PubMed ID: 8862553
TITLE: **Autocatalytic** processing of pro-papaya **proteinase** IV is prevented by crowding of the active-site cleft.
AUTHOR: Baker K C; Taylor M A; Cummings N J; Tunon M A; Worboys K A; Connerton I F
CORPORATE SOURCE: Department of Food Macromolecular Science, Institute of Food Research, Reading Laboratory, UK.
SOURCE: PROTEIN ENGINEERING, (1996 Jun) 9 (6) 525-9.
Journal code: PR1; 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X78056
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 20000303
Entered Medline: 19961209

AB The DNA coding for pro-papaya **proteinase** IV (PPIV) has been cloned and expressed in Escherichia coli. Heterologous expression of the protein, followed by refolding in vitro, yields an enzymatically active pro-enzyme which fails to autodigest to form the mature protein. Mutagenesis of the active site of papain to simulate that of PPIV yields a proenzyme which also fails to autoactivate. Complementary mutagenesis of the pro-region/mature boundary of PPIV, to introduce its own substrate recognition sequence, has, however, produced a pro-enzyme that will **autocatalytically** cleave. This is the first report of enzymatic activity in a recombinant pro-cysteine **proteinase**, and the first time that such a protein has been shown to fail to **autocatalytically** cleave because of its stringent substrate specificity.

L6 ANSWER 7 OF 39 MEDLINE
ACCESSION NUMBER: 96337518 MEDLINE
DOCUMENT NUMBER: 96337518 PubMed ID: 8743567
TITLE: Purification and refolding of recombinant human proMMP-7 (pro-matrilysin) expressed in Escherichia coli and its characterization.
AUTHOR: Itoh M; Masuda K; Ito Y; Akizawa T; Yoshioka M; Imai K; Okada Y; Sato H; Seiki M
CORPORATE SOURCE: Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Setsunan University, Osaka.
SOURCE: JOURNAL OF BIOCHEMISTRY, (1996 Apr) 119 (4) 667-73.
Journal code: HIF; 0376600. ISSN: 0021-924X.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610

ENTRY DATE: Entered STN: 19961106
Last Updated on STN: 20000303
Entered Medline: 19961024

AB Human matrix metalloproteinase-7 (MMP-7 = matrilysin) was overproduced in Escherichia coli as a recombinant zymogen (31 kDa), the C-terminus of which bears artificial hexa-histidines. Most of the enzyme was isolated from the insoluble fraction of the cell lysate and purified by a single step using Ni-NTA resin after solubilization of the precipitates with 8 M urea solution. The resin-bound recombinant protein was refolded into a form that is activatable by p-amino-phenylmercuric acetate in an **autocatalytic** manner. The activated enzyme cleaved a synthetic peptide substrate at the reported site for MMP-7. Digestion of carboxymethylated transferrin (a natural substrate of MMP-7) by the recombinant **proteinase** generated fragments with the same peptide map as in the case of native purified MMP-7. The **autocatalytic** activation and enzyme reaction were entirely dependent on the presence of calcium and zinc ions. The enzyme activity to cleave carboxymethylated transferrin was inhibited by tissue inhibitors of metalloproteinases-1 and -2, MMP-specific inhibitors. The activity of the recombinant MMP-7 was also inhibited by a synthetic peptide derived from a part of the cysteine switch that maintains the zymogen in an inactive state. Thus, we report here a simple means of preparing a large quantity of recombinant proMMP-7 that can be used to study the activation mechanism and to screen synthetic inhibitors.

L6 ANSWER 8 OF 39 MEDLINE
ACCESSION NUMBER: 96180012 MEDLINE
DOCUMENT NUMBER: 96180012 PubMed ID: 8609620
TITLE: Selection of Streptomyces griseus **protease** B mutants with desired alterations in primary specificity using a library screening strategy.
AUTHOR: Sidhu S S; Borgford T J
CORPORATE SOURCE: Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1996 Mar 29) 257 (2) 233-45.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199605
ENTRY DATE: Entered STN: 19960605
Last Updated on STN: 20000303
Entered Medline: 19960530

AB Streptomyces griseus **protease** B (SGPB) has primary specificity for large hydrophobic residues. The **protease** is secreted in a promature form, and **autocatalytic** removal of the propeptide is essential for activity. We genetically substituted the P1 Leu at the promature junction of SGPB with Phe, Met, or Val and monitored expression levels in Escherichia coli. Substitution with Phe had no effect on active SGPB production; substitution with Met or Val abolished proteolytic activity. An E. coli expression library containing 29,952 possible SGPB mutants was constructed with variations at seven sites involved in conferring primary specificity. A rapid, visual screening strategy was used to detect active **protease** secretion. The expression library was screened, in conjunction with the different promature junction sequences, for those variants producing increased proteolytic activity. The sequences of the isolated mutant genes were determined; the substrate specificities and thermostabilities of the corresponding **protease** were investigated. Mutants isolated from the screen with the wild-type promature junction exhibited substrate specificities and thermostabilities similar to wild-type. The screen with Phe at the promature junction P1 site resulted in the isolation of mutant proteases with increased thermostabilities (up to an order of magnitude increase in half-life at 55 degrees C), while a **protease** with broad substrate specificity was isolated from Val screen. Proteases isolated from the screen with Met at the promature junction P1 site exhibited dramatic increases in activity towards a synthetic substrate with Met at P1 site. The results suggests that the substrate specificity of recombinant SGPB is constrained by the sequence of the promature junction; active **protease** production is dependent on the efficiency of the self-processive promature junction cleavage. With an efficient screening strategy, this relationship can be used to isolate catalytically active proteases with desired specificities engineered at the promature junction.

L6 ANSWER 9 OF 39 MEDLINE
ACCESSION NUMBER: 95252581 MEDLINE
DOCUMENT NUMBER: 95252581 PubMed ID: 7766173
TITLE: High level secretion by Saccharomyces cerevisiae of human apolipoprotein E as a **fusion** to Rhizomucor rennin.
AUTHOR: Nomura N; Yamada H; Matsubara N; Horinouchi S; Beppu T

CORPORATE SOURCE: Department of Biotechnology, University of Tokyo, Japan.
SOURCE: BIOSCIENCE, BIOTECHNOLOGY, AND BIOCHEMISTRY, (1995 Mar) 59 (3) 382-7.
Journal code: BDP; 9205717. ISSN: 0916-8451.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: B
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950809
Last Updated on STN: 19950809
Entered Medline: 19950608

AB As the first step for production of human apolipoprotein E (hApoE) in Saccharomyces cerevisiae, the hApoE cDNA was cloned in Escherichia coli, on the basis of the nucleotide sequence reported previously. When the hApoE cDNA including its pre-sequence-encoding region was expressed under the control of the GAL7 promoter, no protein immunoreactive with anti-hApoE antibody was detected either in the culture medium or inside the cells. For efficient production and secretion of hApoE in S. cerevisiae, the mature hApoE-encoding region was fused to the prepro-sequence region of Rhizomucor rennin (MPR) and to the whole MPR gene including its prepro- and mature-MPR regions. When the **fusion** gene consisting of the prepro-sequence-encoding region and hApoE regions was expressed in S. cerevisiae, no protein reactive with the anti-hApoE antibody was detected in any fraction of the yeast cells, probably due to rapid degradation of the hApoE protein by yeast proteases. On the other hand, when hApoE was expressed as a **fusion** to the whole MPR protein, a considerable amount of the fused protein was secreted into the medium. The prepro-sequence of MPR was correctly processed from the fused protein in the medium by **autocatalytic** activity of MPR and by a **protease**(s) of the host cell.(ABSTRACT TRUNCATED AT 250 WORDS)

L6 ANSWER 10 OF 39 MEDLINE
ACCESSION NUMBER: 95113879 MEDLINE
DOCUMENT NUMBER: 95113879 PubMed ID: 7814421
TITLE: Hepsin, a putative membrane-associated serine **protease**, activates human factor VII and initiates a pathway of blood coagulation on the cell surface leading to thrombin formation.
AUTHOR: Kazama Y; Hamamoto T; Foster D C; Kisiel W
CORPORATE SOURCE: Department of Pathology, University of New Mexico School of Medicine, Albuquerque 87131.
CONTRACT NUMBER: HL35246 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Jan 6) 270 (1) 66-72.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950217
Last Updated on STN: 20000303
Entered Medline: 19950203

AB Previous studies have shown that hepsin is a putative membrane-associated serine **protease** that is required for cell growth (Torres-Rosado, A., O'Shea, K. S., Tsuji, A., Chou, S.-H., and Kurachi, K. (1993) Proc. Natl. Acad. Sci. U.S. A. 90, 7181 7185). In the present study, we have transfected baby hamster kidney (BHK) cells with a plasmid containing the cDNA for human hepsin and examined these cells for their ability to activate several blood coagulation factors including factors X, IX, VII, prothrombin, and protein C. Little, if any, proteolytic activation of factors X, IX, prothrombin, or protein C was observed when these clotting factors were incubated with hepsin-transfected cells. On the other hand, hepsin-transfected cells proteolytically activated significant concentrations of human factor VII in a time- and calcium-dependent manner, whereas essentially no activation of factor VII was observed in BHK cells transfected with plasmid lacking the cDNA for hepsin. The factor VII activating activity in the hepsin-transfected BHK cell line was confined exclusively to the total membrane fraction and was inhibited > 95% by antibody raised against a **fusion** protein consisting of maltose-binding protein and the extracellular domain of human hepsin. An active site factor VII mutant, S344A factor VII, was cleaved as readily as plasma-derived factor VII by hepsin-transfected cells, indicating that factor VII was not converted to factor VIIa **autocatalytically** on the cell surface. In contrast, an activation cleavage site factor VII mutant, R152E factor VII, was not cleaved by hepsin-transfected cells, suggesting that factor VII and S344A factor VII were activated on these cells by cleavage of the Arg152-Ile153 peptide bond. In the copresence of factor VII and factor X, hepsin-transfected BHK cells supported the formation of factor Xa. In addition, in the copresence of factor VII, factor X, and prothrombin, hepsin-transfected BHK cells supported the

formation of thrombin. These results strongly suggest that membrane-associated hepsin converts zymogen factor VII to factor VIIa, which in turn, is capable of initiating a coagulation pathway on the cell surface that ultimately leads to thrombin formation.

L6 ANSWER 11 OF 39 MEDLINE
ACCESSION NUMBER: 94148857 MEDLINE
DOCUMENT NUMBER: 94148857 PubMed ID: 8106397
TITLE: The roles of propeptide in maturation and secretion of Npr **protease** from Streptomyces.
AUTHOR: Chang S C; Chang P C; Lee Y H
CORPORATE SOURCE: Institute of Biochemistry, National Yang-Ming Medical College, Taipei, Taiwan, Republic of China.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Feb 4) 269 (5) 3548-54.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 19940330
Last Updated on STN: 20000303
Entered Medline: 19940318

AB The cloned npr gene of Streptomyces cacaoi encodes a 60-kDa protein (prepro-Npr) consisting of a typical secretory signal peptide, a propeptide (22 kDa), and the 35-kDa mature metalloprotease (Npr). The maturation of Npr occurs extracellularly via an **autocatalytic** cleavage of the secreted intermediate pro-Npr (Chang, P.C., and Lee, Y.-H.W. (1992) J. Biol. Chem. 267, 3952-3958). In this study, we investigated the roles of the propeptide in the maturation and secretion of Npr. Partial deletion of the propeptide region while leaving the signal peptide and the mature Npr sequence intact all led to abolishment of Npr activity and caused concomitant slight and transient accumulation of low molecular weight forms of Npr or pro-Npr derivatives extracellularly. The intact propeptide and its truncated form alone could be secreted into the medium if their NH2 termini were directly fused with the signal peptide sequence of Npr. However, similar **fusion** of the mature **protease** domain to the signal peptide without the propeptide sequence completely abolished the Npr production intracellularly and extracellularly. All these results demonstrate that the propeptide plays an important role in maturation and secretion of Npr **protease**, as in the case of alpha-lytic **protease** and subtilisin. In addition, our data suggest that an intact propeptide region is essential for the formation of mature active Npr, but not for the secretion of Npr and its derivatives. This distinguishes the maturation and secretion of S. cacaoi Npr from those of other propeptide-containing bacterial serine proteases and thermolysin-like **protease**.

L6 ANSWER 12 OF 39 MEDLINE
ACCESSION NUMBER: 94063506 MEDLINE
DOCUMENT NUMBER: 94063506 PubMed ID: 8244023
TITLE: Inactivation of a yeast transactivator by the fused HIV-1 **proteinase**: a simple assay for inhibitors of the viral enzyme activity.
AUTHOR: Murray M G; Hung W; Sadowski I; Das Mahapatra B
CORPORATE SOURCE: Schering-Plough Research Institute, Kenilworth, NJ 07033-0539.
SOURCE: GENE, (1993 Nov 30) 134 (1) 123-8.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940201
Last Updated on STN: 19940201
Entered Medline: 19940105

AB The human immunodeficiency virus type 1 (HIV-1) **proteinase** (PR) and its flanking sequences have been fused in frame between the DNA-binding domain and the transcription-activation domain of the yeast protein, GAL4. As has been shown before with the 3C **proteinase** of Coxsackie virus B3 (CVB3) [Das Mahapatra et al., Proc. Natl. Acad. Sci. USA 89 (1992) 4159-4162], the GAL4::PR **fusion** protein retains its GAL4 function, providing the PR is inactive. When PR is active, its **autocatalytic** activity in the hybrid protein is shown to inactivate the transactivation function of GAL4. This provides a simple assay to monitor PR activity. A dose-dependent effect of a potent PR-specific inhibitor is demonstrated in this system and illustrates the sensitivity of the assay. The assay is used for high throughput screening to identify novel inhibitors of the viral PR, and provides a method to generate and analyze mutants and revertants of the PR.

L6 ANSWER 13 OF 39 MEDLINE
ACCESSION NUMBER: 93290929 MEDLINE
DOCUMENT NUMBER: 93290929 PubMed ID: 8512750
TITLE: Human endogenous retroviral element K10 (HERV-K10) encodes a full-length gag homologous 73-kDa protein and a functional **protease**.
AUTHOR: Mueller-Lantzsch N; Sauter M; Weiskircher A; Kramer K; Best B; Buck M; Grasser F
CORPORATE SOURCE: Abteilung Virologie, Universitatskliniken des Saarlandes, Homburg, Germany.
SOURCE: AIDS RESEARCH AND HUMAN RETROVIRUSES, (1993 Apr) 9 (4) 343-50.
Journal code: ART; 8709376. ISSN: 0889-2229.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930806
Last Updated on STN: 20000303
Entered Medline: 19930720

AB The gag-homologous region of the human endogenous retrovirus K10 (HERV-K10) was amplified by PCR from human genomic DNA and was analyzed by DNA cloning, sequencing, and expression of open reading frames in the prokaryotic pATH expression system. The analysis of genomic DNA of three donors provided evidence that HERV-K10 genes contain an open reading frame of 1966 bp spanning the entire gag-homologous region. In the prokaryotic system the entire reading frame of the HERV-K10 gag gene could be expressed as a **fusion** protein exhibiting a molecular weight of about 110,000. In addition, when the gag-homologous region and the adjacent HERV-K10 **protease** gene were prokaryotically expressed, we observed a Gag-**protease fusion** protein that exhibited specific autoproteolytic activities and processing of HERV-K10 Gag protein. By introducing deletions on the right end of the putative **protease** gene an **autocatalytic** site could be localized within 300 bp of the putative HERV-K10 **protease** gene. For the first time, these results provide evidence that the HERV-K10 encodes a full-length Gag protein and a functional **protease**.

L6 ANSWER 14 OF 39 MEDLINE
ACCESSION NUMBER: 93234439 MEDLINE
DOCUMENT NUMBER: 93234439 PubMed ID: 8386363
TITLE: Site-directed mutagenesis of the putative active site residues of 3C **proteinase** of coxsackievirus B3: evidence of a functional relationship with trypsin-like serine proteinases.
AUTHOR: Miyashita K; Kusumi M; Utsumi R; Katayama S; Noda M; Komano T; Satoh N
CORPORATE SOURCE: Central Research Laboratories, Maruishi Pharmaceutical Co., Ltd., Osaka, Japan.
SOURCE: PROTEIN ENGINEERING, (1993 Feb) 6 (2) 189-93.
Journal code: PR1; 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 19930604
Last Updated on STN: 20000303
Entered Medline: 19930514

AB Picornavirus 3C proteinases (3Cpro) are cysteine proteinases but recent sequence analyses have shown that they are related to trypsin-like serine proteinases. Two models of 3Cpro structure have been presented. Both models indicate that residues His40 and Cys147 are members of the catalytic triad but the models differ in the designation of the third member of the catalytic triad, which is assigned as either Glu71 or Asp85. To test the importance of these four residues in the catalytic activity of 3Cpro of coxsackievirus B3, a member of the enterovirus subgroup of the picornavirus family, single amino acid substitutions were introduced at each of the four sites. All of these mutations resulted in the reduction or inactivation of **autocatalytic** cleavage of the 3C precursor protein expressed in Escherichia coli, suggesting that all of these residues are essential for the proteolytic reaction. The substitution of Cys147 with Ala abolished 3Cpro activity while the mutant in which Cys147 was replaced with Ser retained reduced proteolytic activity both in cis and in trans. Our results strongly support the proposal that Cys147 of 3Cpro functions as a nucleophile analogous to Ser195 of trypsin-like serine proteinases.

L6 ANSWER 15 OF 39 MEDLINE
ACCESSION NUMBER: 93054787 MEDLINE

DOCUMENT NUMBER: 93054787 PubMed ID: 1331110
TITLE: Purification and characterization of the mouse mammary tumor virus **protease** expressed in Escherichia coli.
AUTHOR: Menendez-Arias L; Young M; Oroszlan S
CORPORATE SOURCE: Laboratory of Molecular Virology and Carcinogenesis, National Cancer Institute-Frederick Cancer Research and Development Center, Maryland 21702-1201.
CONTRACT NUMBER: NO1-CO-74101 (NCI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Nov 25) 267 (33) 24134-9.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D10517; GENBANK-D10518; GENBANK-D10519; GENBANK-D10520; GENBANK-D12749; GENBANK-D12750; GENBANK-D12751; GENBANK-D12752; GENBANK-D12753; GENBANK-L01464
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 20000303
Entered Medline: 19921222

AB The mouse mammary tumor virus (MMTV) **protease** gene was cloned into pGEX-2T, an Escherichia coli expression vector containing the glutathione S-transferase coding region of Schistosoma japonicum. The chimeric protein was formed by **fusion** of the glutathione S-transferase with a hexapeptide which contains a thrombin cleavage site, followed by the MMTV **protease**. Affinity chromatography on a glutathione-Sepharose 4B column was used to isolate the chimeric protein. After thrombin cleavage, the glutathione S-transferase and the **protease** were separated by gel filtration chromatography on a Sephadex G-75 column. The overall yield of the **protease** purification procedure was about 1 mg of **protease**/liter of culture, and the specific activity was 380 pmol/min.micrograms of enzyme. Like other retroviral proteases, the MMTV enzyme was active as a dimer, showed maximum activity at pH between 4 and 6, and could be inhibited by pepstatin A and a phosphinic acid derivative HIV-1 **protease** inhibitor. Enzymatic characterization of this **protease** reveals its broad specificity, showing a clear preference for the oligopeptide substrate mimicking the cleavage site at the amino-terminal end of the capsid protein (kcat/Km = 9725.5 M-1.s-1). The chimeric protein was also an active dimer and showed a similar Km (17 microM) for such an oligopeptide, although its kcat was about 10 times smaller. **Autocatalytic** processing of the MMTV **protease** was observed after expression of clones containing the natural cleavage site, as it occurs at the amino-terminal end of the viral **protease**, instead of the thrombin-sensitive sequence.

L6 ANSWER 16 OF 39 MEDLINE
ACCESSION NUMBER: 92341057 MEDLINE
DOCUMENT NUMBER: 92341057 PubMed ID: 1634873
TITLE: **Autocatalytic** activity of the tobacco etch virus NIa **proteinase** in viral and foreign protein sequences.
AUTHOR: Rorrer K; Parks T D; Scheffler B; Bevan M; Dougherty W G
CORPORATE SOURCE: Department of Microbiology, Oregon State University, Corvallis 97331-3804.
SOURCE: JOURNAL OF GENERAL VIROLOGY, (1992 Apr) 73 (Pt 4) 775-83.
Journal code: I9B; 0077340. ISSN: 0022-1317.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920911
Last Updated on STN: 20000303
Entered Medline: 19920821

AB The small nuclear inclusion (NIa) protein of the tobacco etch virus (TEV) is synthesized initially as part of a genome-derived high M(r) precursor. The NIa protein releases itself from this genome-derived precursor by self-cleavage, or an **autocatalytic** processing event. Cleavage between specific glutamine-glycine dipeptides at the N and C termini generates the 430 amino acid or 49,000 M(r) (49K) NIa protein. The requirements of this **autocatalytic** release, or cis cleavage, were examined by constructing gene cassettes encoding the TEV NIa protein which could be ligated into particular locations in cDNA of the TEV genome and also into foreign gene DNA sequences. Using cell-free transcription and translation systems, polyproteins containing TEV NIa sequences were synthesized and assayed for (i) autocatalysis and (ii) the ability of a functional NIa **proteinase**, purified from plant tissue, to cleave

in bimolecular or trans reactions various artificial polyproteins which contained an inactive form of the NIa **proteinase**. The NIa self-cleavage events required an active **proteinase** sequence and a consensus TEV cleavage site sequence at the N and C termini. These results were consistent for NIa protein sequences placed at a foreign TEV cleavage site or in unrelated proteins. Differences were noted in the trans cleavage of these sites.

L6 ANSWER 17 OF 39 MEDLINE
ACCESSION NUMBER: 92330027 MEDLINE
DOCUMENT NUMBER: 92330027 PubMed ID: 1369382
TITLE: Expression and purification of recombinant 3C **proteinase** of Coxsackievirus B3.
AUTHOR: Miyashita K; Kusumi M; Utsumi R; Komano T; Satoh N
CORPORATE SOURCE: Central Research Laboratories, Maruishi Pharmaceutical Co., Ltd., Osaka, Japan.
SOURCE: BIOSCIENCE, BIOTECHNOLOGY, AND BIOCHEMISTRY, (1992 May) 56 (5) 746-50.
Journal code: BDP; 9205717. ISSN: 0916-8451.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: B
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19950713
Last Updated on STN: 20000303
Entered Medline: 19920814
AB We have cloned various lengths of coxsackievirus B3 cDNA encompassing the region encoding the 3C **proteinase**, which is essential to the viral replication cycle. Such viral cDNAs were fused in frame to the 5'terminal portion of the lacZ' gene carried on the vector pUC118 to express mature 3C **proteinase** in Escherichia coli. In the E. coli cells containing pCXB108 or pCXB117, constructed for this study, a large amount of 23-kDa protein was synthesized in the presence of IPTG. This protein was purified and was shown to be intact 3C **proteinase**. These data suggest that 3C **proteinase**, expressed as a part of a **fusion** protein, was active in E. coli and released itself from the precursor **fusion** protein by **autocatalytic** cleavage.

L6 ANSWER 18 OF 39 MEDLINE
ACCESSION NUMBER: 92260662 MEDLINE
DOCUMENT NUMBER: 92260662 PubMed ID: 1316486
TITLE: Complementation studies with Rous sarcoma virus gag and gag-pol polyprotein mutants.
AUTHOR: Oertle S; Bowles N; Spahr P F
CORPORATE SOURCE: Department of Molecular Biology, University of Geneva, Switzerland.
SOURCE: JOURNAL OF VIROLOGY, (1992 Jun) 66 (6) 3873-8.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920626
Last Updated on STN: 20000303
Entered Medline: 19920616
AB Avian retroviruses (with the notable exception of spleen necrosis virus) express their **protease** (PR) both in their gag and their gag-pol polyprotein precursors, in contrast to other retroviruses, notably, the mammalian retroviruses, in which PR is encoded in the gag-pol polyprotein or in a separate reading frame as a gag-pro product. The consequence is that the avian PR is expressed in stoichiometric rather than catalytic amounts. To investigate the significance of the particular genome organization of the avian retrovirus prototype Rous sarcoma virus, we developed an assay that measures complementation between the gag and the gag-pol polyproteins by expressing them from two different plasmids in transfected cells. By using this assay, we showed that the **protease** PR from the gag-pol polyprotein is capable of **autocatalytic** self-cleavage and -activation when coexpressed with a **protease**-deficient gag protein and that the PR domain has a role in viral particle assembly. Furthermore, this complementation assay can be used to investigate the role of the gag domain in the gag-pol polyprotein by determining whether it can rescue a defect in the gag polyprotein. We report here the results of such an experiment, which studied a mutation in the N terminus of the gag gene.

L6 ANSWER 19 OF 39 MEDLINE
ACCESSION NUMBER: 92230243 MEDLINE
DOCUMENT NUMBER: 92230243 PubMed ID: 1314466
TITLE: Expression of the **protease** gene of equine infectious anemia virus in Escherichia coli: formation of

the mature processed enzyme and specific cleavage of the gag precursor.
AUTHOR: Rushlow K; Peng X X; Montelaro R C; Shih D S
CORPORATE SOURCE: Department of Biochemistry, Louisiana State University, Baton Rouge.
CONTRACT NUMBER: CA49296 (NCI)
SOURCE: VIROLOGY, (1992 May) 188 (1) 396-401.
Journal code: XEA; 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 19920607
Last Updated on STN: 19920607
Entered Medline: 19920515

AB A 620-bp Bg/II restriction fragment containing the putative **protease** coding sequence from equine infectious anemia virus (EIAV) proviral DNA was cloned and expressed in E. coli as a Pol precursor protein. In contrast to the 25-kDa **fusion** protein predicted from the expressed pol sequence, a protein of approximately 10 kDa was generated by apparent **autocatalytic** processing of the Pol precursor. This mature processed protein was detected in transformed cells using an antisera raised against synthetic peptide from the conserved carboxyl-terminal segment of the predicted EIAV **protease** coding sequence. Coexpression of this protein with a 35-kDa EIAV Gag-precursor **fusion** protein resulted in the specific proteolytic processing of the precursor as shown by formation of p26, the major capsid protein of EIAV.

L6 ANSWER 20 OF 39 MEDLINE
ACCESSION NUMBER: 92218393 MEDLINE
DOCUMENT NUMBER: 92218393 PubMed ID: 1559982
TITLE: The sequence, organization, and expression of the major cysteine **protease** (cruzain) from Trypanosoma cruzi.
AUTHOR: Eakin A E; Mills A A; Harth G; McKerrow J H; Craik C S
CORPORATE SOURCE: Department of Pharmaceutical Chemistry, University of California, San Francisco 94143.
CONTRACT NUMBER: GM07175 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Apr 15) 267 (11) 7411-20.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L01675; GENBANK-L01676; GENBANK-L01677; GENBANK-M83679; GENBANK-M83680; GENBANK-M83681; GENBANK-M83724; GENBANK-M84342; GENBANK-X53708; GENBANK-X53709
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 19920529
Last Updated on STN: 20000303
Entered Medline: 19920512

AB The complete sequence of the gene encoding the major cysteine **protease** from Trypanosoma cruzi is reported. The amino acid sequence predicted from the gene sequence aligns well with members of the papain family of cysteine proteases, suggesting the name cruzain. The sequence is most closely related to the cysteine **protease** of Trypanosoma brucei (59.3%) and the murine cathepsin L (42.2%). At least six copies of the gene are present in the genome and are organized in a tandem array of copies which are identical in all restriction endonuclease sites tested. The gene appears to be expressed in all developmental stages of T. cruzi with mRNA levels approximately 2-fold higher in the intracellular amastigote form. A copy of the T. cruzi gene was expressed in bacteria as an inactive, insoluble **fusion** polypeptide to approximately 5% of the total cell protein. The **fusion** protein was readily purified, solubilized in urea, and successfully refolded to produce a polyprotein which processed **autocatalytically** to yield approximately 1 mg of active **protease** per 3 g of wet cell paste. The processed form of the recombinant **protease** has an NH2-terminal sequence identical to that of the mature form of the **protease** purified from T. cruzi (Murta, A. C. M., Persechini, P. M., Souto-Padron, T., de Souza, W., Guimaraes, J. A., and Scharfstein, J. (1990) Mol. Biochem. Parasitol. 43, 27-38; Cazzulo, J. J., Couso, R., Raimondi, A., Wernstedt, C., and Hellman, U. (1989) Mol. Biochem. Parasitol. 33, 33-42). This suggests that the recombinant **protease** possesses the requisite specificity and activity to correctly process the proform of the **protease** in vitro. Kinetic assays with peptide substrates demonstrate that the substrate specificity and kinetic parameters for the recombinant **protease** are consistent with

those of the endogenous **protease**. The proteolytic activity of the recombinant **protease** is enhanced by dithiothreitol, inhibited by leupeptin, N alpha-p-tosyl-L-lysine chloromethyl ketone and trans-epoxysuccinyl-L-leucylamido(4-guanidino)butane (E-64) but is unaffected by phenylmethylsulfonyl fluoride, pepstatin, and 1,10-phenanthroline. More specifically, the recombinant enzyme was inhibited by benzyloxycarbonyl-Phe-Arg-fluoromethyl ketone, which inhibits replication and differentiation of T. cruzi within mammalian cells in culture.

L6 ANSWER 21 OF 39 MEDLINE
ACCESSION NUMBER: 92015504 MEDLINE
DOCUMENT NUMBER: 92015504 PubMed ID: 1656087
TITLE: cis- and trans-cleavage activities of poliovirus 2A **protease** expressed in Escherichia coli.
AUTHOR: Alvey J C; Wyckoff E E; Yu S F; Lloyd R; Ehrenfeld E
CORPORATE SOURCE: Department of Cellular Biology, University of Utah, School of Medicine, Salt Lake City 84132.
CONTRACT NUMBER: AI 12387 (NIAID)
AI 27914 (NIAID)
SOURCE: JOURNAL OF VIROLOGY, (1991 Nov) 65 (11) 6077-83.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199111
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 20000303
Entered Medline: 19911114

AB The poliovirus **protease**, 2Apro, was produced in Escherichia coli from plasmids that encode a **fusion** protein consisting of the N-terminal portion of the bacterial TrpE protein linked to poliovirus 2Apro. This **fusion** protein underwent efficient **autocatalytic** cleavage at the N terminus of 2Apro, generating the mature **protease**. Extracts of bacteria expressing 2Apro induced the specific cleavage of the p220 subunit of the eukaryotic translation initiation factor 4F, similar to the 2Apro-mediated reaction that occurs in poliovirus-infected HeLa cells. A portion of the poliovirus polyprotein containing the 2Apro cleavage site at the P1/P2 junction was produced by translation of cDNA transcripts in rabbit reticulocyte lysates and then tested as a substrate for 2Apro-mediated cleavage. The protein was partially cleaved by 2Apro in trans. Finally, a 16-amino-acid synthetic peptide, representing the P1/P2 junction sequence, was analyzed as a substrate for 2Apro. The peptide was labeled with fluorescein at a lysine residue to facilitate its detection. Recombinant 2Apro cleaved the synthetic peptide into two half-peptide molecules which were resolved by high-pressure liquid chromatography. Direct sequence analysis of the isolated peptide products demonstrated that cleavage occurred at the expected tyrosine-glycine pair. A rapid cleavage assay for 2Apro activity on the synthetic peptide was developed, using separation of the fluorescein-labeled 8-amino-acid product from the 16-residue substrate by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels.

L6 ANSWER 22 OF 39 MEDLINE
ACCESSION NUMBER: 92011682 MEDLINE
DOCUMENT NUMBER: 92011682 PubMed ID: 1918024
TITLE: Autoflavinylation of apo6-hydroxy-D-nicotine oxidase.
AUTHOR: Brandsch R; Bichler V
CORPORATE SOURCE: Biochemisches Institut, Freiburg, Federal Republic of Germany.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1991 Oct 5) 266 (28) 19056-62.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199111
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19980206
Entered Medline: 19911108

AB 6-Hydroxy-D-nicotine oxidase (6-HDNO) was expressed in Escherichia coli JM109 cells from the recombinant plasmid pAX-6-HDNO as a beta-galactosidase-6-HDNO **fusion** protein. Affinity chromatography of the **fusion** protein on p-aminobenzyl-1-thio-beta-galactopyranoside-agarose and subsequent digestion with **protease** Xa yielded highly purified apo6-HDNO. Incubation of the purified protein with [14C]FAD demonstrated that flavinylation of apo6-HDNO proceeds **autocatalytically**. Phosphorylated three-carbon compounds such as glycerol-3-P, which are known to stimulate the formation of the histidyl (N3)-(8 alpha) FAD between apo6-HDNO and FAD

(Brandsch, R., and Bichler, V. (1989) Eur. J. Biochem. 182, 125-128), could be replaced in their action by high concentrations of glycerol (45%) or sucrose (20%). These substances apparently induced and stabilized a conformational state of the apoenzyme compatible with covalent attachment of FAD. In the absence of glycerol the apoenzyme readily lost the ability to form holoenzyme at temperatures above 30 degrees C. Holoenzyme formation protected the 6-HDNO polypeptide from this thermal denaturation. Autoflavinylation of 6-HDNO was inhibited by the sulfhydryl reagents dithionitrobenzoate or N-ethylmaleimide. Inhibition was prevented by mercaptoethanol or FAD, but not 6-hydroxy-D-nicotine, the substrate of the holoenzyme. A cysteine-thiol group may therefore be involved in reactions leading to the covalent attachment of FAD to apo6-HDNO. When flavinylation of apo6-HDNO proceeded under anaerobic conditions, the amount of incorporation of [14C]FAD into the polypeptide was indistinguishable from reactions performed in the presence of O2. None of the FAD-derivatives (8-demethyl-FAD, 8-chloro-FAD, and 5-deaza-FAD) could replace FAD in holoenzyme formation. The failure of covalent attachment of 5-deaza-FAD to apo6-HDNO is in agreement with the assumption that the quinone methide form of the isolloxazine ring is an intermediate in the flavinylation reaction.

L6 ANSWER 23 OF 39 MEDLINE
ACCESSION NUMBER: 91333017 MEDLINE
DOCUMENT NUMBER: 91333017 PubMed ID: 1651406
TITLE: Flavivirus enzyme-substrate interactions studied with chimeric proteinases: identification of an intragenic locus important for substrate recognition.
AUTHOR: Preugschat F; Lenches E M; Strauss J H
CORPORATE SOURCE: Division of Biology, California Institute of Technology, Pasadena 91125.
CONTRACT NUMBER: AI20612 (NIAID)
SOURCE: JOURNAL OF VIROLOGY, (1991 Sep) 65 (9) 4749-58.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199109
ENTRY DATE: Entered STN: 19911006
Last Updated on STN: 20000303
Entered Medline: 19910919

AB The proteins of flaviviruses are translated as a single long polyprotein which is co- and posttranslationally processed by both cellular and viral proteinases. We have studied the processing of flavivirus polyproteins in vitro by a viral **proteinase** located within protein NS3 that cleaves at least three sites within the nonstructural region of the polyprotein, acting primarily **autocatalytically**. Recombinant polyproteins in which part of the polyprotein is derived from yellow fever virus and part from dengue virus were used. We found that polyproteins containing the yellow fever virus cleavage sites were processed efficiently by the yellow fever virus enzyme, by the dengue virus enzyme, and by various chimeric enzymes. In contrast, dengue virus cleavage sites were cleaved inefficiently by the dengue virus enzyme and not at all by the yellow fever virus enzyme. Studies with chimeric proteinases and with site-directed mutants provided evidence for a direct interaction between the cleavage sites and the proposed substrate-binding pocket of the enzyme. We also found that the efficiency and order of processing could be altered by site-directed mutagenesis of the proposed substrate-binding pocket.

L6 ANSWER 24 OF 39 MEDLINE
ACCESSION NUMBER: 91254282 MEDLINE
DOCUMENT NUMBER: 91254282 PubMed ID: 1904218
TITLE: Expression of functional beta-galactosidase containing the coxsackievirus 3C **protease** as an internal **fusion**.
AUTHOR: Windheuser M G; Dwyer S; Dasmahapatra B
CORPORATE SOURCE: Schering-Plough Research, Bloomfield, New Jersey 07003.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1991 May 31) 177 (1) 243-51.
Journal code: 9Y8; 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199107
ENTRY DATE: Entered STN: 19910728
Last Updated on STN: 20000303
Entered Medline: 19910705

AB Alpha complementation of beta-galactosidase (beta gal) is intracistronic and requires interaction between the alpha donor region (residues 3-41) and alpha acceptor fragment (produced by M15). We have constructed two

plasmids which direct the synthesis of hybrid beta gal: coxsackievirus proteins in Escherichia coli. One plasmid, pBD1045, encodes an enzymatically active 3C **protease** of coxsackievirus B3 fused between the amino-terminal 79 amino acids of beta gal (containing the alpha donor region) and amino acids 80 to 1023 (alpha acceptor region). A second plasmid, pBD1043 encodes an inactive 3C **protease** and results in a **fusion** of 260 coxsackievirus amino acids between residues 79 and 80 of the beta gal monomer. Both hybrid proteins expressed by these constructs have beta-galactosidase activity regardless of whether the viral **protease** (183 amino acids) is **autocatalytically** cleaved out of the chimeric protein (pBD1045) or remains as part of a **fusion** protein (pBD1043). The implications of these results for structural flexibility of the complemented beta-galactosidase enzyme are discussed.

L6 ANSWER 25 OF 39 MEDLINE
ACCESSION NUMBER: 91053175 MEDLINE
DOCUMENT NUMBER: 91053175 PubMed ID: 2241167
TITLE: Human immunodeficiency viral **protease** is catalytically active as a **fusion** protein: characterization of the **fusion** and native enzymes produced in Escherichia coli.
AUTHOR: Boutelje J; Karlstrom A R; Hartmanis M G; Holmgren E; Sjogren A; Levine R L
CORPORATE SOURCE: Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892.
SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1990 Nov 15) 283 (1) 141-9.
Journal code: 6SK; 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199012
ENTRY DATE: Entered STN: 19910208
Last Updated on STN: 19970203
Entered Medline: 19901224
AB Processing of the gag and pol gene precursor proteins of retroviruses is essential for the production of mature infectious virions. The processing is directed by a viral **protease** that itself is part of these precursors and is presumed to cleave itself **autocatalytically**. To facilitate study of this process, the **protease** was produced as a **fusion** protein in Escherichia coli. In this construct, the 10,793-Da **protease** was preceeded by two copies of a modified IgG binding domain derived from protein A. The IgG binding domain was linked to the **protease** by an Asp-Pro peptide bond which could not be cleaved by the viral **protease**. A dimer of the 25,400-Da **fusion** protein was catalytically active, specifically cleaving a substrate peptide at the correct Tyr-Pro bond. Thus, the **fusion** protein could serve as a model of the viral gag-pol polyprotein. The finding that the **fusion** protein was catalytically active supports the suggestion that a gag-pol dimer can initiate a proteolytic cascade after budding of the immature virus. The **fusion** protein also provided a source of authentic **protease**. The **protease** was released from the **fusion** construct by incubation with formic acid, cleaving the Asp-Pro linkage which had been inserted between the IgG binding domain and the **protease**.

L6 ANSWER 26 OF 39 MEDLINE
ACCESSION NUMBER: 90324953 MEDLINE
DOCUMENT NUMBER: 90324953 PubMed ID: 2197372
TITLE: Detection of the trans activity of the plum pox virus NIa-like **protease** in infected plants.
AUTHOR: Himmler G; Frank S; Steinkellner H; Ruker F; Mattanovich D; Katinger H W
CORPORATE SOURCE: Institute of Applied Microbiology, University of Agriculture, Wien, Austria.
SOURCE: JOURNAL OF GENERAL VIROLOGY, (1990 Jul) 71 (Pt 7) 1623-5.
Journal code: I9B; 0077340. ISSN: 0022-1317.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199008
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900824
AB The NIa-like protein of plum pox virus is a **protease** with high sequence specificity that is **autocatalytically** released from the viral polyprotein. In order to determine whether the **protease** is active in trans we constructed a **fusion** protein consisting of

the C-terminal region of the plum pox virus polyprotein and the staphylococcal Protein A. The authentic **protease** recognition sequence Asn-Val-Val-Val-His-Gln-Ala occurs in the centre of this protein **fusion**. This protein was cleaved specifically by extracts of plum pox virus-infected plants due to the strong activity of the viral **protease** making it a useful tool for diagnostic purposes.

L6 ANSWER 27 OF 39 MEDLINE
ACCESSION NUMBER: 90281589 MEDLINE
DOCUMENT NUMBER: 90281589 PubMed ID: 2191498
TITLE: The **proteinase** polypeptide of adenovirus serotype 2 virions.
AUTHOR: Anderson C W
CORPORATE SOURCE: Biology Department, Brookhaven National Laboratory, Upton, New York 11973.
CONTRACT NUMBER: U01 AI26049-02 (NIAID)
SOURCE: VIROLOGY, (1990 Jul) 177 (1) 259-72.
Journal code: XEA; 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199007
ENTRY DATE: Entered STN: 19900824
Last Updated on STN: 19970203
Entered Medline: 19900718

AB The Ad2 **proteinase**, which is thought to be encoded by a 23-kDa open reading frame located at the end of the L3 family of late mRNAs, is expressed poorly even late after infection. To obtain sufficient **proteinase** for biochemical characterization, a DNA fragment containing the 23-kDa open reading frame was cloned into plasmids that permit efficient expression in Escherichia coli. Polyclonal antiserum specific for the Ad2 **proteinase** was produced by immunizing rabbits with a **fusion** protein that included the entire **proteinase** open reading frame, and this antiserum was used to show that the product of the 23-kDa reading frame is assembled into virions. Bacterial products corresponding to the complete 204 amino acid **proteinase** reading frame, to a 9 amino acid **proteinase** deletion, and to a **proteinase fusion** protein of 227 amino acids were used to determine the size of the **proteinase** polypeptide in Ad2 virions and in infected HeLa cell extracts. A single **proteinase** polypeptide that migrated during SDS-polyacrylamide gel electrophoresis with the 204 amino acid recombinant **proteinase** was detected in wild-type and H2ts1 virions, and in infected cell extracts. Immunoblot titrations showed that a wild-type Ad2 virus particle contains about 10 **proteinase** polypeptides; an H2ts1 virion has approximately fivefold less **proteinase**. In virions, the **proteinase** was associated primarily with the virus core. The 204 amino acid **proteinase** produced in E. coli permitted cleavage of the major core protein precursor, P-VII, to mature, authentic VII, but the **proteinase** deletion lacking 9 amino acids from near the amino-terminus was inactive. These results are inconsistent with **autocatalytic** processing of the Ad2 **proteinase** as was reported by Chatterjee and Flint (1987, Proc. Natl. Acad. Sci. USA 84, 714-718).

L6 ANSWER 28 OF 39 MEDLINE
ACCESSION NUMBER: 90269618 MEDLINE
DOCUMENT NUMBER: 90269618 PubMed ID: 2189788
TITLE: Adenovirus proteinases: comparison of amino acid sequences and expression of the cloned cDNA in Escherichia coli.
AUTHOR: Houde A; Weber J M
CORPORATE SOURCE: Department of Microbiology, Faculty of Medicine, University of Sherbrooke, Quebec, Canada.
SOURCE: GENE, (1990 Apr 16) 88 (2) 269-73.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199007
ENTRY DATE: Entered STN: 19900810
Last Updated on STN: 20000303
Entered Medline: 19900712

AB Adenoviruses (Ad) synthesize serine-center endoproteinases (AdEPs) responsible for maturation cleavages within the virus particle. Many questions regarding these enzymes remain unanswered because previous studies utilized crude cells or viral lysates as the enzyme source. Here, we report on the comparison of the amino acid (aa) sequences of several AdEPs and on the expression of the cDNA of the Ad2Ep in Escherichia coli. The AdEPs consist of about 200 aa and their size is around 23 kDa. Among the seven sequences known, 60% of aa were strictly conserved. The usual

serine **proteinase** active site sequence, GDSGG, is absent. The recombinant Ad2EP, produced by an inducible vector as a protein-A **fusion** product is capable of **autocatalytic** cleavage, and of cleaving its natural viral substrates as well as foreign proteins. Therefore, other viral proteins or mammalian specific post-translational modifications are not required for enzyme activity.

L6 ANSWER 29 OF 39 MEDLINE
ACCESSION NUMBER: 90059863 MEDLINE
DOCUMENT NUMBER: 90059863 PubMed ID: 2684630
TITLE: Cauliflower mosaic virus produces an aspartic **proteinase** to cleave its polyproteins.
AUTHOR: Torruella M; Gordon K; Hohn T
CORPORATE SOURCE: Friedrich Miescher-Institut, Basel, Switzerland.
SOURCE: EMBO JOURNAL, (1989 Oct) 8 (10) 2819-25.
Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199001
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 20000303
Entered Medline: 19900102

AB Cauliflower mosaic virus (CaMV), a plant pararetrovirus, produces polyproteins from its adjacent genes for the coat protein (ORF IV) and for enzymatic functions (ORF V). The N-terminal domain of the latter gene includes a sequence showing homology to the active site of other retroviral and acid proteases. We have now shown that this domain does indeed produce a functional aspartic **protease** that can process both the polyproteins. Mutations in the putative active site abolished virus infectivity. In transient expression studies in protoplasts, the N-terminal domain of ORF V was able to free active CAT enzyme from a precursor containing an N-terminal **fusion** of a portion of ORF IV. The junction between the two domains of this artificial polyprotein comprised sequences from the ORF IV product that had previously been shown to include a proteolytic processing site. The **protease** mutants were not able to free active CAT enzyme from this precursor. Direct analysis of cleavage at the same site in the ORF IV product using proteins expressed in Escherichia coli revealed the expected products. In vitro translation of a synthetic transcript covering ORF V was used to study the **autocatalytic** cleavage of the ORF product. Pulse-chase experiments showed that the 80 kd initial translation product was processed to yield a N-terminal doublet of polypeptides of 22 and 20 kd apparent mol. wt, which cover the **protease** domain. The mutants in the active site were not processed.

L6 ANSWER 30 OF 39 MEDLINE
ACCESSION NUMBER: 89052670 MEDLINE
DOCUMENT NUMBER: 89052670 PubMed ID: 2461297
TITLE: Active site mutagenesis of the AIDS virus **protease** and its alleviation by trans complementation.
AUTHOR: Le Grice S F; Mills J; Mous J
CORPORATE SOURCE: Central Research Units, F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland.
SOURCE: EMBO JOURNAL, (1988 Aug) 7 (8) 2547-53.
Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198901
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19890104

AB Replacement of the putative active site Asp residue of cloned HIV-1 **protease** with Ala yields a molecule incapable of **autocatalytic** processing. Similarly, **protease**/reverse transcriptase and **protease**/reverse transcriptase/endonuclease polyproteins containing the same mutation accumulate as enzymatically inert polyproteins. Introduction of a second, wild-type, copy of **protease** in trans alleviates this defect, leading in the case of individually cloned **protease** to cleavage of the mutant protein, and with the polyprotein mutants to release of the reverse transcriptase and endonuclease polypeptides, the former of which recover enzymatic activity. In related experiments, a similar inhibition and trans-complementation of a genetically engineered gag--**protease** **fusion** protein was observed.

L6 ANSWER 31 OF 39 MEDLINE
ACCESSION NUMBER: 89012222 MEDLINE
DOCUMENT NUMBER: 89012222 PubMed ID: 3050149

TITLE: Processing of in vitro-synthesized gag precursor proteins of human immunodeficiency virus (HIV) type 1 by HIV **proteinase** generated in Escherichia coli.
AUTHOR: Krausslich H G; Schneider H; Zybarth G; Carter C A; Wimmer E
CORPORATE SOURCE: Department of Microbiology, State University of New York, Stony Brook 11794-8621.
CONTRACT NUMBER: AI 25993 (NIAID)
RR 05736 (NCRR)
SOURCE: JOURNAL OF VIROLOGY, (1988 Nov) 62 (11) 4393-7.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198811
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 20000303
Entered Medline: 19881121

AB We expressed the gag and **proteinase** regions of human immunodeficiency virus (HIV) type 1 by transcription and translation in vitro. A synthetic RNA spanning the gag and pro domains gave primarily the unprocessed capsid precursor pr53. Efficient cleavage of this precursor was observed when the gag and pro domains were placed in the same translational reading frame, yielding equimolar amounts of the gag protein and of **proteinase** (PR). Expression of HIV type 1 PR in Escherichia coli as a **fusion** protein gave rapid **autocatalytic** processing to an HIV-specific protein of approximately 11 kilodaltons. HIV PR generated in E. coli specifically induced cleavage of the HIV capsid precursor, whereas deletion of the carboxy-terminal 17 amino acids of the **proteinase** rendered it inactive. Inhibitor studies showed that the enzyme was insensitive to inhibitors of serine and cysteine proteinases and metalloproteinases and was inhibited only by a very high concentration (1 mM) of pepstatin A.

L6 ANSWER 32 OF 39 MEDLINE
ACCESSION NUMBER: 89005069 MEDLINE
DOCUMENT NUMBER: 89005069 PubMed ID: 3049075
TITLE: Partial purification and substrate analysis of bacterially expressed HIV **protease** by means of monoclonal antibody.
AUTHOR: Hansen J; Billich S; Schulze T; Sukrow S; Moelling K
CORPORATE SOURCE: Max-Planck-Institut fur Molekulare Genetik, Abt. Schuster, Berlin, FRG.
SOURCE: EMBO JOURNAL, (1988 Jun) 7 (6) 1785-91.
Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198811
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 20000303
Entered Medline: 19881107

AB Retroviruses code for a specific **protease** which is essential for polyprotein precursor processing and viral infectivity. The HIV-specific **protease** has been predicted to be an aspartic **protease** which is located at the amino terminus of the pol gene. We have prepared several constructs for bacterial expression of the **protease**. Two of them span the whole **protease** region and result in its **autocatalytic** activation. Analysis of the dynamics of this activation indicates a two-step process which starts at the carboxy terminus and ends at the amino terminus of the **protease**. The activated **protease** is a molecule of 9 kd as evidenced by monoclonal antibody in immunoblot analysis. A construct in which the carboxy terminus of the **protease** is deleted results in a stable, enzymatically inactive 27-kd protein which proved useful as substrate since it contains one of the predicted cleavage sites. The stability of this protein indicates that the carboxy-terminal sequences of the **protease** are essential for its activity and its **autocatalytic** activation. The **protease** which is very hydrophobic was solubilized by acetone treatment and passaged over ultrogel and propylagarose columns for partial purification. It elutes as a dimer and tends to aggregate. It is inhibited by pepstatin A in agreement with its expected active site and its theoretical classification as aspartic **protease**. Cleavage of the gag precursor results in the mature capsid protein, p17. The **protease** does not, however, cleave the denatured 27-kd substrate or the denatured gag precursor. Therefore its specificity appears to be not solely sequence- but also conformation-dependent. This property needs to be taken into account for the development of **protease** inhibitors for therapy of AIDS.

L6 ANSWER 33 OF 39 MEDLINE
ACCESSION NUMBER: 88179537 MEDLINE
DOCUMENT NUMBER: 88179537 PubMed ID: 2833011
TITLE: A poliovirus mutant defective for self-cleavage at the COOH-terminus of the 3C **protease** exhibits secondary processing defects.
AUTHOR: Kean K M; Agut H; Fichot O; Wimmer E; Girard M
CORPORATE SOURCE: Unite de Virologie Moleculaire, UA CNRS 545, Institut Pasteur, Paris, France.
CONTRACT NUMBER: AI 15122 (NIAID)
CA 28146 (NCI)
SOURCE: VIROLOGY, (1988 Apr) 163 (2) 330-40.
Journal code: XEA; 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198805
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19880502
AB By in vitro recombination between the wild-type full-length infectious cDNA of poliovirus and a clone generated by the construction of a cDNA bank from a chemically derived temperature-sensitive plurimutant, we obtained a mutant cDNA with a T to C change at nucleotide 5658. This mutation replaces the isoleucine at residue 74 of the viral **protease** 3C by a threonine. The mutant virus recovered after transfection exhibited a small-plaque phenotype, and was deficient for viral RNA synthesis. Both these defects were more marked at 39 than at 37 degrees. The mutation was introduced into a bacterial plasmid which expresses the 3C **protease** along with its flanking **autocatalytic** cleavage sites. Analysis of the cleavage products expressed in Escherichia coli provided direct evidence that the modification impaired cleavage at the COOH-terminus of 3C. Cleavage at this same site was partially defective in mutant virus-infected HeLa cells, reducing the production of mature 3C and the viral replicase, 3D. Cleavage of P1, the precursor to the capsid polypeptides, was apparently unaffected by this defect, whereas cleavage events within the P2 region of the genome occurred inefficiently. This is indicative of differential strategies for 3C-specific cleavage events in vivo.

L6 ANSWER 34 OF 39 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:24689 CAPLUS
DOCUMENT NUMBER: 128:189727
TITLE: Characterization of drug resistant retroviral **proteinase** mutants
AUTHOR(S): Good, Valerie M.; Anderson, Marie M.; Baker, Jon J.; Moloudi, Adi; James, Colin H.; Wilderspin, Andrew F.
CORPORATE SOURCE: Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, London, WC 1N 1AX, UK
SOURCE: Biochem. Soc. Trans. (1997), 25(4), S633
CODEN: BCSTB5; ISSN: 0300-5127
PUBLISHER: Portland Press Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Maturation of virally encoded polyproteins is essential for the propagation of HIV and other retroviruses. The viral **proteinase** is initially expressed as a 180 kDa gag-pol **fusion** protein, FP, which subsequently undergoes **autocatalytic** cleavage and yields the structural proteins and enzymes. The mature **proteinase** is made up of two identical 99 amino acid monomers, which each contribute one catalytic aspartate residue to the active site. Extended therapeutic use of the drug saquinavir selects for resistant virus with mutations at positions 90 (L .fwdarw. M) and 48 (G .fwdarw. V). SIV **proteinase** mutants L90M and G48V were created by site-directed mutagenesis. Complete self-processing of the wild-type and L90M FPs yielded the expected 11 kDa **proteinase** monomer. However, G48V **proteinase** contained an addnl. 15 kDa protein that could be explained by incomplete processing at the C-terminus of the gene 10-G48V FP. The 15 kDa protein could not be induced to self-process to the 11 kDa form. Thus, the 15 kDa G48V **proteinase** FP appears to adopt a cleavage resistant state.

L6 ANSWER 35 OF 39 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:601800 CAPLUS
DOCUMENT NUMBER: 115:201800
TITLE: Cleavage of RT/RNase H by HIV-1 **protease** and analysis of substrate cleavage sites in vitro
AUTHOR(S): Moelling, Karin; Nawrath, M.; Schulze, T.; Pavlitzkova, L.; Soucek, M.; Budt, K. H.; Pearl, L. H.; Knoop, M. T.; Kay, J.; Kruft, V.
CORPORATE SOURCE: Max Planck-Inst. Mol. Genet., Berlin, D-1000/33, Fed. Rep. Ger.

SOURCE: Retroviral Proteases (1990), 19-29. Editor(s): Pearl, Laurence H. Macmillan: Basingstoke, UK.
CODEN: 57FQAH
DOCUMENT TYPE: Conference
LANGUAGE: English

AB The pol gene of HIV-1 virus expressed in bacteria undergoes **autocatalytic** processing which results in the generation of the p66 reverse transcriptase (RT)/RNase H, the p32 endonuclease, and the p9 aspartic **protease**. The partially purified **protease** generates in vitro from the p66 mol. a p66/p51 heterodimer, typical of the normally obsd. RT/RNase H, and a p15 C-terminal fragment. A synthetic peptide, AETF'YVD, derived from the p51/p15 junction is cleaved by the **protease** in vitro and may represent the natural cleavage site. Here, the RT/RNase H activities assocd. with p66 and p51 and p66/p51 heterodimers were detd. after renaturation of these proteins from polyacrylamide gels. The p66/p51 heterodimers exhibited .apprx.8-fold higher RT and RNase H activities than each of the individual subunits alone suggesting that heterodimers are of biol. relevance. **Protease** activity was monitored by 3 assays, by cleavage of denatured ovalbumin, of the MS2-gag **fusion** protein, or synthetic peptides. Several synthetic peptides representing various potential **protease** cleavage sites and modifications thereof were analyzed in vitro for their efficiency of cleavage by the **protease**. One synthetic peptide representing the natural N-terminal cleavage site of the **protease** was modified by several amino acid substitutions in order to characterize the specificity of the **protease**. Several of the peptides as well as cerulenin and acetyl-pepstatin were analyzed as **protease** inhibitors.

L6 ANSWER 36 OF 39 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1990:153052 CAPLUS
DOCUMENT NUMBER: 112:153052
TITLE: Cloning and expression of **protease** P2A gene of human rhinovirus 2 and substrates for assay of its activity
INVENTOR(S): Sommergruber, Wolfgang; Fessl, Friederike; Kuechler, Ernst; Blaas, Dieter; Skern, Timothy; Zorn, Manfred; Duechler, Markus; Kowalski, Heinrich; Volkmann, Peter; Maurer-Fogy, Ingrid
PATENT ASSIGNEE(S): Boehringer Ingelheim International G.m.b.H., Fed. Rep. Ger.
SOURCE: Eur. Pat. Appl., 37 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 321973	A2	19890628	EP 1988-121480	19881222
EP 321973	A3	19900613		
EP 321973	B1	19931103		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
DE 3743848	A1	19890706	DE 1987-3743848	19871223
DE 3825118	A1	19900125	DE 1988-3825118	19880723
AT 96839	E	19931115	AT 1988-121480	19881222
ES 2059482	T3	19941116	ES 1988-121480	19881222
JP 02031678	A2	19900201	JP 1988-325729	19881223
US 5149783	A	19920922	US 1988-288894	19881223

PRIORITY APPLN. INFO.: DE 1987-3743848 19871223
DE 1988-3825118 19880723
EP 1988-121480 19881222

AB DNA encoding a **fusion** protein comprising a **protease** and a polypeptide which can be **autocatalytically** cleaved from the **protease** is described. This constitutes a system in which potentially therapeutic inhibitors of the **protease** can be tested and the functionally important regions of the protein analyzed by in vitro mutagenesis. A cDNA clone encoding the human rhinovirus 2 **protease** region of the viral polyprotein including dipeptides cleaved to activate the enzyme substrate was cloned in Escherichia coli as a **fusion** gene with MS2 polymerase sequences. A deletion deriv. of this sequence that was not a **protease** substrate is also prepd. Plasmids encoding the active and inactive forms of the **protease** were expressed in vitro in a prokaryotic transcription/translation system and the expression analyzed by SDS-PAGE. The plasmid encoding the active form of the **protease** showed a no. of bands consistent with accurate processing and activation of the precursor. The deletion mutant, lacking a functionally important arginine residue in this region, showed no evidence of processing.

L6 ANSWER 37 OF 39 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1989:208444 CAPLUS
DOCUMENT NUMBER: 110:208444
TITLE: Polypeptide 2A of human rhinovirus type 2: identification as a **protease** and characterization by mutational analysis
AUTHOR(S): Sommergruber, Wolfgang; Zorn, Manfred; Blaas, Dieter; Fessl, Friederike; Volkmann, Peter; Maurer-Fogy, Ingrid; Pallai, Peter; Merluzzi, Vincent; Matteo, Martha
CORPORATE SOURCE: Ernst Boehringer Inst. Arzneimittelforsch., Vienna, A 1120, Austria
SOURCE: Virology (1989), 169(1), 68-77
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Evidence is presented that the protein 2A of human rhinovirus serotype 2 (HRV2) is a **protease**. On expression of the VP1-2A region of HRV2 in Escherichia coli, protein 2A was capable of acting on its own N-terminus; derived exts. specifically cleaved a 16 amino acid oligopeptide corresponding to the sequence at the cleavage site. Cleavage of the oligopeptide substrate provided a convenient in vitro assay system. Deletion expts. showed that removal of 10 amino acids from the carboxy terminus inactivated the enzyme. Site-directed mutagenesis identified an essential arginine close to the C-terminus and showed that the enzyme was sensitive to changes in the putative active site. This anal. supports the hypothesis that 2A belongs to the group of sulfhydryl proteases, although sequence comparisons indicate that the putative active site of HRV2 2A is closely related to that of the serine proteases.

L6 ANSWER 38 OF 39 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1988:586333 CAPLUS
DOCUMENT NUMBER: 109:186333
TITLE: In vivo and in vitro autoprocessing of human immunodeficiency virus **protease** expressed in Escherichia coli
AUTHOR(S): Giam, Chou Zen; Boros, Imre
CORPORATE SOURCE: Coll. Med., Univ. Nebraska, Omaha, NE, 68105, USA
SOURCE: J. Biol. Chem. (1988), 263(29), 14617-20
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The viral-specific **protease** of human immunodeficiency virus (HIV) has been expressed as a lacZ-**protease fusion** protein. This **fusion** protein contains **protease** cleavage sites at the gag/**protease** and **protease** /reverse transcriptase junctions and undergoes autoprocessing in vivo when expressed in Escherichia coli. The purified lacZ-**protease fusion** protein precursors also exhibited autoproteolytic activity in vitro. One cleavage product of the autoprocessing reactions is a 10-kDa protein that cross-reacts with peptide antisera prepd. against the putative **protease** sequence. Consistent with the notion that HIV **protease** is an acid **protease**, its autoproteolytic activity is inhibited in alk. buffers and by pepstatin A. The in vivo and in vitro autocleavage assays for HIV **protease** together with the overprodn. of the **protease** should facilitate design and testing of therapeutic agents that inhibit gag-pol polyprotein processing and HIV virion maturation.

L6 ANSWER 39 OF 39 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95349890 EMBASE
DOCUMENT NUMBER: 1995349890
TITLE: A cysteine **protease** is a target for the enzyme structure-based design of antiparasitic drugs.
AUTHOR: Eakin A.E.; McKerrow J.H.; Craik C.S.
CORPORATE SOURCE: Department of Biochemistry, Puerto Rico Univ. School of Medicine, P.O. Box 365067,San Juan 00936-5067, Puerto Rico
SOURCE: Drug Information Journal, (1995) 29/SUPPL. (1501S-1517S).
ISSN: 0092-8615 CODEN: DGIJB
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Proteases have been shown to be factors in the pathogenicity of many parasitic diseases, either by inducing tissue damage and facilitating invasion or by enabling the parasites to salvage metabolites from host proteins. To study genes encoding cysteine proteases of parasites, a general method for cloning fragments of thiol **protease** genes was developed using the polymerase chain reaction (PCR) with degenerate

oligonucleotide primers. Subsequently, a PCR-amplified gene fragment of the protozoan parasite, Trypanosoma cruzi, was used to isolate a full-length gene encoding a cysteine **protease**. At least six copies of the gene are organized in the genome as a tandem array. The high degree of sequence identity with the papain family of enzymes suggested the name 'cruzain.' A copy of the gene was expressed in bacteria as an inactive, insoluble **fusion** polypeptide. Subsequently, the **fusion** protein was solubilized in urea and refolded to produce a polyprotein which processed **autocatalytically** to yield active, recombinant enzyme. This expression method generated recombinant **protease** of sufficient quality and quantity for crystallization. Diffraction quality crystals of recombinant cruzain, inactivated with a peptide inhibitor shown to block growth of the parasites in infected human cells, have been produced and characterized. The studies presented herein will provide insight into the mechanism of action and structure of cruzain and may enable the development of specific inhibitors for antiparasitic chemotherapy in the treatment of Chagas' disease.